# A multi-immune gene prognostic model for evaluation of survival and prognosis of colorectal cancer

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# Abstract

**Objective:** We aimed to construct a multi-immune gene model for the prognosis of colorectal cancer. This study would not only provide important clinical data for the evaluation of survival and prognosis of colorectal cancer, but provide insights into the tumor immune mechanisms.

**Methods:** Colorectal cancer gene expression and clinicopathological data were downloaded from the TCGA database, and then we performed gene expression analysis to obtain differentially expressed genes. In addition, we downloaded immune genes from the ImmPort immune gene database, and obtained differentially expressed immune genes after intersection with the differentially expressed colorectal cancer genes. We further performed survival analysis of the differential immune genes to obtain prognosis-related genes, which were used to construct a multi-immune gene prognostic model. We then analyzed the impact of the prognostic model risk score on the survival of colorectal cancer patients through survival analysis, using ROC analysis. In addition, we performed risk curve analysis to validate the accuracy of the prognostic model risk score in assessing the prognosis of colorectal cancer, and also conducted independent prognostic analysis. Finally, we analyzed the correlation between the immune genes, and transcription factors as well as immune cells.

**Results:** Our analysis showed that prognosis of the high-risk group as evaluated by the immune gene prognosis model risk score was poor ($P$<0.001). The prognostic model risk score could accurately classify the colorectal patients and has high accuracy in the analysis of prognosis of colorectal cancer (AUC=0.861). Our data demonstrated a certain correlation between the immune genes, transcription factors and immune cells.

**Conclusions:** The constructed prognostic model could accurately assess the prognosis and survival of patients with colorectal cancer. Immune genes might regulate malignant progression of tumors by modulating the production of transcription factors and immune cells. This study demonstrated the influence of immune factors on the prognosis of colorectal cancer and provided a reference for further studies evaluating the role of immunity in the development of colorectal cancer.


# Introduction

Colorectal cancer (CRC) is the third most malignant tumor that threatens human health, and its high incidence (1096601 new cases worldwide every year, 6.1% of all cancers) and mortality (551269 new cases worldwide every year, 5.8% of all cancers) present major global public health challenges [1]. Although there has been significant progress in studies evaluating molecular mechanisms of CRC, it remains a leading cause of death due to its high rate of metastasis and recurrence. Therefore, there is an urgent need for identification of a novel tool that could be used to predict prognosis in the treatment of CRC [2]. Most studies have constructed prognostic models based on clinicopathological characteristics (e.g, tumor size, tumor number, lymph nodes, and vascular invasion) and single-molecule biomarkers (e.g,

carcinoembryonic antigen CEA, carbohydrate antigens CA199, CH24, and CA242) [3, 4]. Nevertheless, there are still no reliable indicators that could be used accurately predict the prognosis of CRC.

With intensive studies on the pathogenesis and development of CRC, there is significant data available on the role of immunity in malignant progression of CRC. As has been demonstrated, immune responses confer a bidirectional effect on the development of cancer. Surprisingly, whereas cancerous tissues can be eliminated by the immune system under normal conditions, the immune system has been shown to fuel carcinogenesis in some contexts [5, 6]. Owing to the heterogeneity among the CRCs, genomic instability enables CRC to generate distinct cell populations under clonal selection [7]. Distinct immune properties exist among the cell populations. For example, mutated tumor cells acquire the ability to proliferate indefinitely by evading host immune system, and the molecular events that make the tumor cells heterogeneous might promote cancer initiation and progression [8, 9].

Recent studies have shown that prognostic models developed as a result of combination of several genes could significantly improve the accuracy of predicting prognosis, especially in breast, prostate and liver cancers. However, data on the role of immune related genes in the development of CRC remain scant [10–12]. Here, we used prognosis related immune genes to establish a prognostic model. The study employed a combination of multiple immune genes in the prediction of prognosis in CRC. Besides, interactions of immune cells, transcription factors and immune genes in the CRCs were also analyzed. The use of a wide spectrum of genes increased the sensitivity of the model, which provides a reliable indicator for the treatment and prognosis of CRC as well as a reference for the study of immune responses in CRC.

# Methods And Materials

### ##Screening for differentially expressed genes

CRC gene expression datasets (containing 41 normal colorectal tissue samples and 473 CRC tissue samples) and clinicopathological features were downloaded from the Cancer Genome Atlas Database (TCGA), and the expression datasets were analyzed using the R software "limma" package to identify genes that were differentially expressed in CRC [13].

### ##Screening for differentially expressed immune genes

A total of 2498 immune related genes in CRC were downloaded from the immune gene database analysis portal (immport), and the immune genes that were differentially expressed in CRC were filtered out using Perl software to the intersection of differential genes and immune genes [14].

### ##Screening for prognosis related immune genes

Colorectal clinicopathological data from the TCGA database were collated followed by removal of the data with incomplete pathology. Patient survival times (containing a total of 395 survival data) were

merged with the differentially expressed gene data and then survival analysis was performed using the R software "" survival "" package to screen out prognosis related immune genes in CRC.

## ##Screening for differential transcription factors

We downloaded a total of 318 tumor associated transcription factors from transcription factor (TF) sites (http://www.cistrome.org/). The TFs which were differentially expressed in CRC were obtained after intersection with the differentially expressed genes [15].

## ##Construction of an immune gene prognostic model for colorectal cancer

Multivariate Cox analysis of differentially expressed immune genes in CRC was performed to screen for differentially expressed immune genes that could serve as independent prognostic risk factors for CRC. The risk coefficient of each risk gene to patient prognosis was calculated, and then the immune gene prognostic model was constructed based on the risk coefficient. The immune gene risk model was calculated using = (coefficientmrna1 × Expression of mrna1) + (coefficientmrna2 × Expression of mrna2) + (coefficientmrnan × Expressing mrnan) [16].

## ##Validation of the immune gene prognostic model risk score in colorectal cancer

Patients were divided into high-risk group (risk score > median risk score) and low-risk group (risk score < median risk score) according to the prognostic model risk score. We employed the R software "" survival "" package for the analysis of survival using the risk score. Then, the prognostic value of the risk score was estimated using the R software "survivalroc" package to draw the ROC curve, and the prognostic value of the prognostic model for patients was evaluated using the area under the curve (AUC). AUC = 1, better prediction, AUC = [0.85, 0.95], predicted very well, AUC = [0.7, 0.85], predicting effect in general, AUC = [0.5, 0.7], lower effect size.

## ##Immune gene prognostic model and independent prognostic analysis of patients' clinicopathological characteristics

The prognostic model risk scores and clinicopathological characteristics were combined with patient survival time.  Univariate and multivariate Cox analyses were then performed on the pooled data to evaluate the independent risk of the risk score and each clinicopathological feature in the prognostic model. Univariate Cox analysis indicated that there was correlation between the risk factors and patient prognosis. The multivariate independent analysis demonstrated that the risk factors could independently indicate patient prognosis.

## ##Correlation between immune genes and transcription factors

The correlation analysis between the differential transcription factors and differential immune genes was performed using R software. Results were filtered according to the correlation coefficient R| R| < 0.3

showed lack of correlation, 0.3 < |r| < 0.5 a low degree of correlation, 0.5 < |r| < 0.8 a moderate correlation, while 0.8 < |r| showed high correlation.

### ##Correlation between prognostic models and immune cells in colorectal cancer

We employed the tumor immune resource website (https://cistrome.shinyapps.io/timer/, TIMER) to download the content of immune cells in the respective CRC cases in the TCGA database. The immune cell data and prognostic model data were arcuated and cases with both sets of data were filtered out. R software was then used to perform correlation analysis between the immune cell content and risk score of the prognostic model.

# Results

### ##Screening for differentially expressed genes in colorectal cancer

CRC gene expression datasets (containing 41 normal colorectal tissue samples and 473 CRC tissue samples) were downloaded from the TCGA database. The R software "limma " package was used to analyze and profile the expression of genes in CRC, which was screened with a |LogFC| > 1, P < 0.05. Our analysis showed that a total of 6478 genes were differentially expressed in CRC. Out of the total genes, 1716 were downregulated while 4762 genes were upregulated. Using the R software "" pheatmap "" package, we generated a heatmap (Fig.1a) and volcano plot (Fig.1b) of the differentially expressed genes.

### ##Screening for differentially expressed immune genes in colorectal cancer

A total of 2498 CRC associated immune genes were downloaded from the immune gene database analysis portal (immport). We then used Perl software to perform an intersection between the immune genes and the differentially expressed genes in CRC. A total of 467 differentially expressed immune genes in CRC were filtered out, and the "" pheatmap "" package in R software was used to draw a heat map (Fig. 2a), volcano plot (Fig. 2B) of the differentially expressed immune genes.

### ##Screening of prognosis related immune genes in colorectal cancer

Clinicopathological data of CRC were downloaded from the TCGA database, and patient survival times were then merged with the differentially expressed immune genes in CRC. Using the "" survival "" package in R software, a total of 50 prognosis related immune genes, including 11 low-risk ratio genes and 39 high-risk ratio genes, were filtered out by univariate Cox analysis, and then a forest plot was constructed (Fig. 3). Hazard ratio (HR) [HR = hazard function H1 (T) for the exposed group / H2 (T) for the non-exposed group, and t refers to the same time points].

### ##Screening of differential transcription factors in colorectal cancer

A total of 318 tumor associated transcription factors were downloaded from the transcription factor (TF) site (http://www.cistrome.org/). To obtain transcription factors that were differentially expressed in CRC, tumor related transcription factors were intersected with the differentially expressed genes in CRC. The analysis showed that 68 transcription factors were differentially expressed, of which 23 were downregulated while 45 were upregulated. We used the "" pheatmap "" package in R software to draw a heat map (Fig. 4a), a volcano plot (Fig. 4b).

## Construction of immune gene prognostic model in colorectal cancer

Multivariate Cox analysis of the differentially expressed immune genes in CRC was performed using the "" survival "" package ins R software. A total of 18 differentially expressed immune genes, of which 4 genes were negatively correlated with prognosis and 14 genes were positively correlated with prognosis, as well as risk coefficients of model genes were selected (Table 1). To construct the model, we used an immune gene model risk score = (coefficientmrna1 × Expression of mrna1) + (coefficientmrna2 × Expression of mrna2) + (coefficientmrnan × Expressed mrnan).

## Validation of the immune gene prognostic model risk score for use in patients with colorectal cancer

Patients were divided into high-risk group (risk value > median risk value) and low-risk group (risk value < median risk value), according to the prognostic model risk score. The risk score of the patients was referenced to the survival analysis performed by the "" survival "" package in R software (Fig. 5a). The "" survivalroc "" package was used to analyze the predictive value of the risk score for the prognosis of patients. We plotted the ROC curve (Fig. 5b) and calculated the area under the curve (AUC), AUC = 1, better prediction, AUC = [0.85, 0.95], which predicted well, AUC = [0.7, 0.85], general predictive effect, AUC = [0.5, 0.7], lower effect. To evaluate the value of prognostic models in the assessment of patient outcomes, the "" pheatmap "" package was used to analyze the relationship between each patient's risk score and their survival status, and then the survival status plot (Figure 5C), risk curve (Figure 5d) as well as risk Heatmap (Figure 5e) were plotted. The results demonstrated that the high-risk group had relatively poor prognosis compared with the low-risk group ($P < 0.05$). The prognostic model performed well in the assessment of patient outcome (AUC = 0.861, and patients with a high-risk score had an overall poor prognosis. Thus, the model showed a more reliable value for patient outcomes.

## Immune gene prognostic model and independent prognostic analysis of patients' clinicopathological characteristics

The prognostic model risk scores and clinicopathological characteristics of the CRC patients were combined, and then univariate (Fig. 6a) and multivariate (Fig. 6b) Cox analyses were performed to analyze independent prognostic roles of the prognostic model and clinicopathological characteristics of the CRC patients, as shown by forest plots. The results showed that the immune gene prognostic model, the TNM stage and patient age could be used as independent risk factors to evaluate the prognosis of the patients.

##**Transcription factor and immune gene correlation analysis in colorectal cancer**

To further investigate the correlation between transcription factors and immune genes in CRC, we used R software to analyze the correlation between the differentially expressed transcription factors and immune genes in CRC. The data were filtered a correlation coefficient, $|r| > 0.4$, $P < 0.001$. In addition, we used Cytoscape software to generate a visual regulatory network diagram of the transcription factors and immune genes (Fig. 7). The results showed that Slit2, INHBA, sema3g and Plcg2 immune genes were significantly correlated with transcription factors, while immune genes such as CCL28 and CD1b were poorly correlated with transcription factors. Transcription factors such as lmc2, IKZF1, and IRF4 were strongly associated with immune genes, while KLF4, CDK2, and EZH2 were poorly correlated with the immune genes. Thus, we speculated that transcription factors might be playing an important role in CRC by interacting with immune genes.

##**Correlation between prognostic models and immune cells in colorectal cancer**

We used the tumor immune resource website (https://cistrome.shinyapps.io/timer/, timer) to download the content of immune cells in the respective CRC cases in the TCGA database. Our analysis showed that the contents of the immune cells were correlated with the risk of prognostic model, as demonstrated by scatter plots (Fig. 8a-e). The data showed that the risk score was not significantly correlated with B cells (Fig. 8a) but was positively correlated with cd4-t cells (Fig. 8b), cd8-t cells (Fig. 8C), dendritic cells (Fig. 8D), macrophages (Fig. 8e), and neutrophils (Fig. 8F) with $|r| > 0.1$, $P < 0.05$. The analysis showed that immune genes can affect the generation of immune cells and synergistically promote colorectal carcinogenesis.

# Discussion

According to the "tumor immunoediting" doctrine, tumors are a result of immune escape [17]. A tumor cell is an abnormal cell that manifests as a genetic mutation that overexpresses oncogenes. In theory, immune cells can eliminate abnormal cells by recognizing these mutations and the aberrantly expressed proteins, thus eradicating the tumor in budding state, a phenomenon referred to as the "immunosurveillance" effect [18]. However, immunosurveillance does not completely abrogate the development of malignancy. Tumors progressively increase in malignancy and eventually undergo extensive metastasis as the disease progresses.

Tumor initiation and development crop from a series of dynamic and complex interaction processes between the immune system and tumor cells[19]. Newly generated tumor cells are highly antigenic and are quickly cleared by the immune system under both nonspecific immune mechanisms (e.g., phagocytic cells, natural killer cells, etc) and specific immune mechanisms (e.g., CD4 + T cells, CD8 + T cells)[20, 21]. Some tumor cells mutate during their interaction with the immune system and overcome the immune 'clearance'. Under the pressure of the immune system, the surviving tumor cells could constantly undergo mutations. Accumulation of mutational effects leads to development of a wide spectrum of phenotypes

by the tumor cells (such as being unable to express MHC molecules, or unable to produce tumor peptides), which aid their escape from destruction by the immune system [22]. In addition, tumor cells can modify their own apoptotic signaling pathways to evade immune-specific tumor cell apoptosis. At the same time, tumors create a microenvironment in which immune suppressive molecules, such as TGF, are released by the tumor cells, and can induce production of regulatory T lymphocytes expressing CTLA-4. This cascade of events exert an inhibitory effect on other immune cells, leading to the development of tolerance by the immune system towards the tumors[23, 24]. At this point, the antitumor mechanisms of the immune system collapses, and there is uncontrolled growth of the tumor cells, leading to tumor metastasis.

In this study, we screened out immune genes related to the prognosis of CRC using TCGA database, and then constructed an immune gene prognostic model. These genes employ different pathways to regulate the function of immune cells, and induce generation of an immune microenvironment for tumorigenesis and progression. For example, CD1b can present lipid antigens in tumor cells to activate reactive T cells (hj1t), which play a protective role in tumor immunity by directly lysing tumor cells using CD8 + T cells [25]. In addition, fibroblast growth factor 2 (FGF2) has a wide range of regulatory effects on cellular biological functions, such as proliferation, angiogenesis, migration, differentiation and repair of injuries. CCL28, as a chemokine with broad antimicrobial activity against gram negative and gram positive bacteria, as well as fungi, acts as a chemoattractant for cells expressing ccr10 and / or CCR3, and plays a dual role in mucosal immunity[27]. The role of CCL28 in linking innate and adaptive immunity exhibits strong homing ability to B and T cells, and coordinates lymphocyte trafficking and functions[28, 29]. Ilc3 is an important component of the intestinal barrier protective system. Intestinal ilc3 neural hubs form a very tightly regulated tissue-specific circuit to induce ilc3 dependent IL-22 cytokine production via the vasoactive intestinal peptide (vip)-vipr2 signaling pathway to control inflammatory intestinal diseases [30]. The role of VIP in immunity could be summarized as regulation of innate and adaptive immunity, including anti-inflammatory effects, regulation of Th1 / Th2 balance, induction of regulatory T cells, and generation of tolerogenic dendritic cells [31, 32].

Our immune gene prognostic model exhibited some advantages in predicting the prognosis of CRC, and the risk score established based on the prognostic model had a reliable prognostic value in patients. Indeed, the prognosis of patients in the high-risk group was poor compared to that in the low-risk group. The prognostic model risk score could serve as an independent risk factor for evaluating the prognosis of patients, which further validated our prognostic model. Furthermore, we demonstrated that transcription factors were differentially expressed in CRC, and had a reciprocal regulatory relationship with immune genes. The immune gene prognostic model risk score had a correlation with immune cells such as cd4-t and cd8-t cells. Thus, we speculate that transcription factors could regulate the prognosis of CRC patients by regulating differential expression of the immune cells to affect production and promote development of tumor cells and metastasis.

We then analyzed the prognostic role of the dynamic immune genes in CRC. Despite our exhaustive and meticulous analysis of the immune genes, transcription factors and immune cells in CRC, and the potentially substantial clinical implications of our results, several questions remain unanswered. First, colorectal carcinogenesis is a multistep, multiprogram, multigenic process. Our prognostic model only involved 18 immune genes, and did not include important differential immune genes, thus might have reduced the performance of the model. Second, to demonstrate the complex mechanisms involved in CRC tumorigenesis and progression, there is need to verify the expression of transcription factors, immune genes and the immune cell content need to be verified in CRC.

# Declarations

### Competing interests

The authors have no conflicts of interest to declare.

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

The datasets analysed during the current study are available in the [The Cancer Genome Atlas] database, [https://portal.gdc.cancer.gov/]


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### Authors’contributions

(I) Conception and design: Bo Hao and Hexin Wen, (II) Administrative support: None, (III) Provision of study materials or patients: Yitong Wang and Mulin Liu, (IV) Collection and assembly of data: Rui Dong

and Shuran Chen, (V) Data analysis and interpretation: Lugen Zuo and Quanwei Qiu, (VI) Manuscript writing: All authors, (VII) Final approval of manuscript: All authors.


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# Tables

Table 1. Screening of immune genes for construction of prognostic models in colorectal cancer

| ID | coef | HR | HR.95L | HR.95H | pvalue |
|---|---|---|---|---|---|
| CD1B | -2.155976693 | 0.115790044 | 0.02232811 | 0.600468834 | 0.01024876 |
| SLC10A2 | 0.782264364 | 2.18641751 | 1.351351517 | 3.537511497 | 0.001440093 |
| FABP4 | 0.008285932 | 1.008320355 | 1.000117572 | 1.016590416 | 0.046792537 |
| FGF2 | 0.337506581 | 1.401448831 | 1.144974262 | 1.715373778 | 0.001065103 |
| CCL28 | -0.095694683 | 0.908741428 | 0.856395586 | 0.964286828 | 0.001570311 |
| IGHG1 | -0.000732798 | 0.999267471 | 0.9985924 | 0.999942998 | 0.033562553 |
| IGHV4-31 | 0.013604819 | 1.013697786 | 1.005661675 | 1.021798113 | 0.000807408 |
| IGKV1-6 | 0.008862377 | 1.008901764 | 1.004108388 | 1.013718023 | 0.000265007 |
| IGKV1-8 | 0.042254831 | 1.043160274 | 1.006934751 | 1.080689048 | 0.019119346 |
| ESM1 | 0.214226484 | 1.238903215 | 1.144090679 | 1.34157301 | 1.34E-07 |
| STC2 | 0.043707547 | 1.044676792 | 0.997685046 | 1.093881885 | 0.062706293 |
| TNFSF12 | 0.071779075 | 1.074417952 | 0.983332525 | 1.17394056 | 0.112265354 |
| UCN | 0.43658071 | 1.547407129 | 1.229882742 | 1.946908222 | 0.000194669 |
| UTS2 | 0.165410499 | 1.179877358 | 0.965107433 | 1.442441051 | 0.106631656 |
| VIP | 0.084268737 | 1.087921219 | 1.044614328 | 1.133023497 | 4.78E-05 |
| GLP2R | -4.61268623 | 0.009925121 | 0.000631273 | 0.156046663 | 0.001032719 |
| IL1RL2 | 0.181334897 | 1.198816592 | 1.038151643 | 1.384346141 | 0.013512859 |
| TRDC | 0.111556883 | 1.118017339 | 1.009457521 | 1.238251976 | 0.032307538 |

Note: The risk coefficient (coef) represents the contribution of genes in the risk score of the model. A negative value indicates that the gene is negatively associated with prognosis, and a positive value indicates that the gene is positively correlated with prognosis.

# Figures

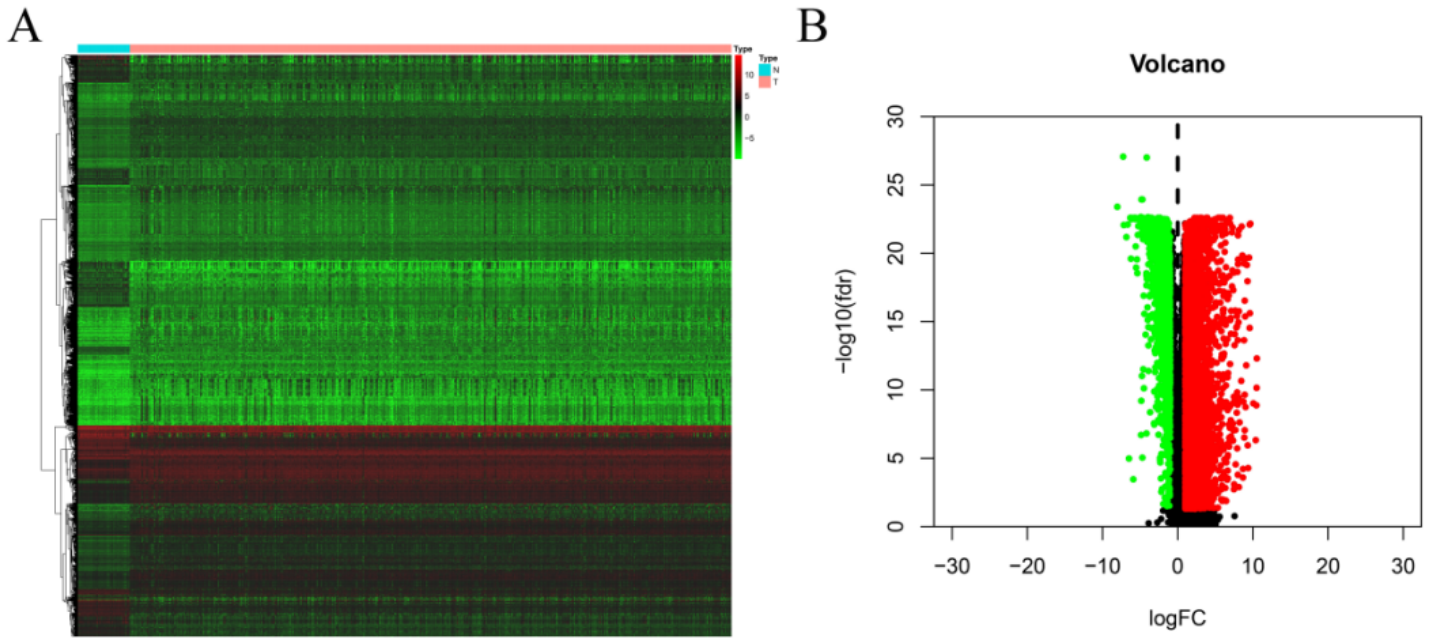



**Figure 1**

Heat map (A) and volcano map (B) showing the differentially expressed genes in colorectal cancer. Red: indicates genes whose expression was up-regulated, green: indicates genes whose expression was down-regulated.

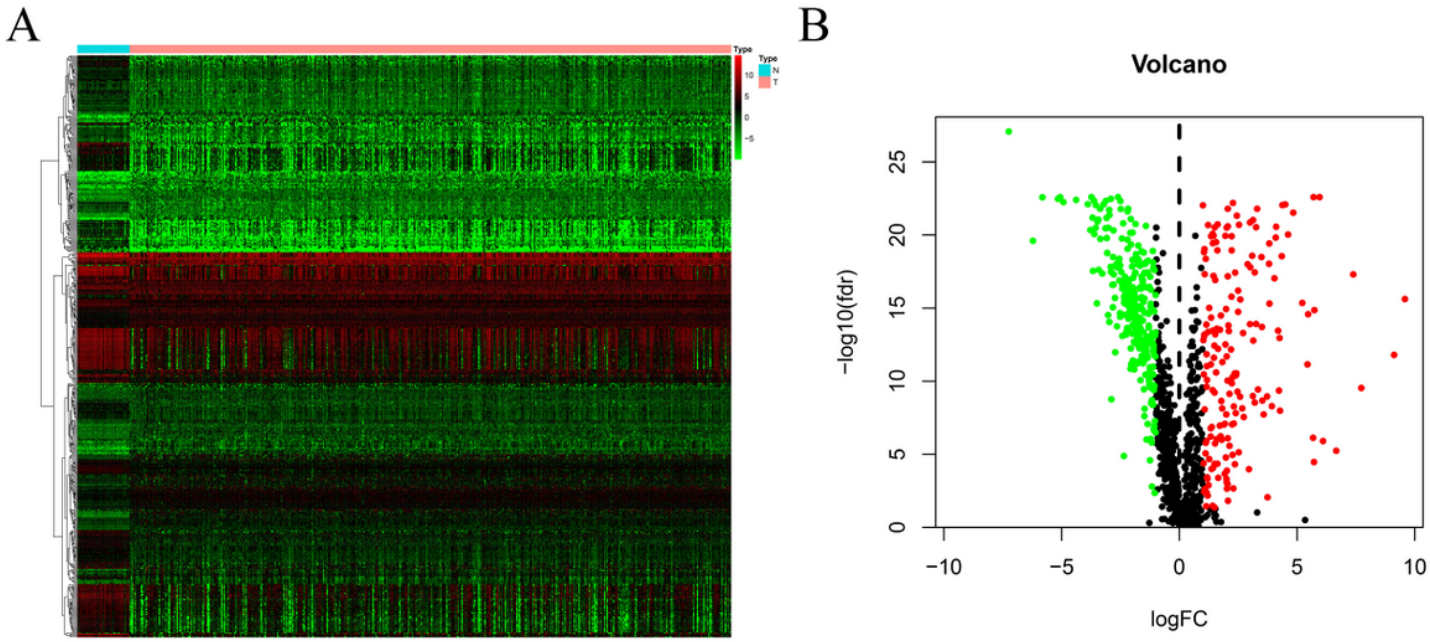
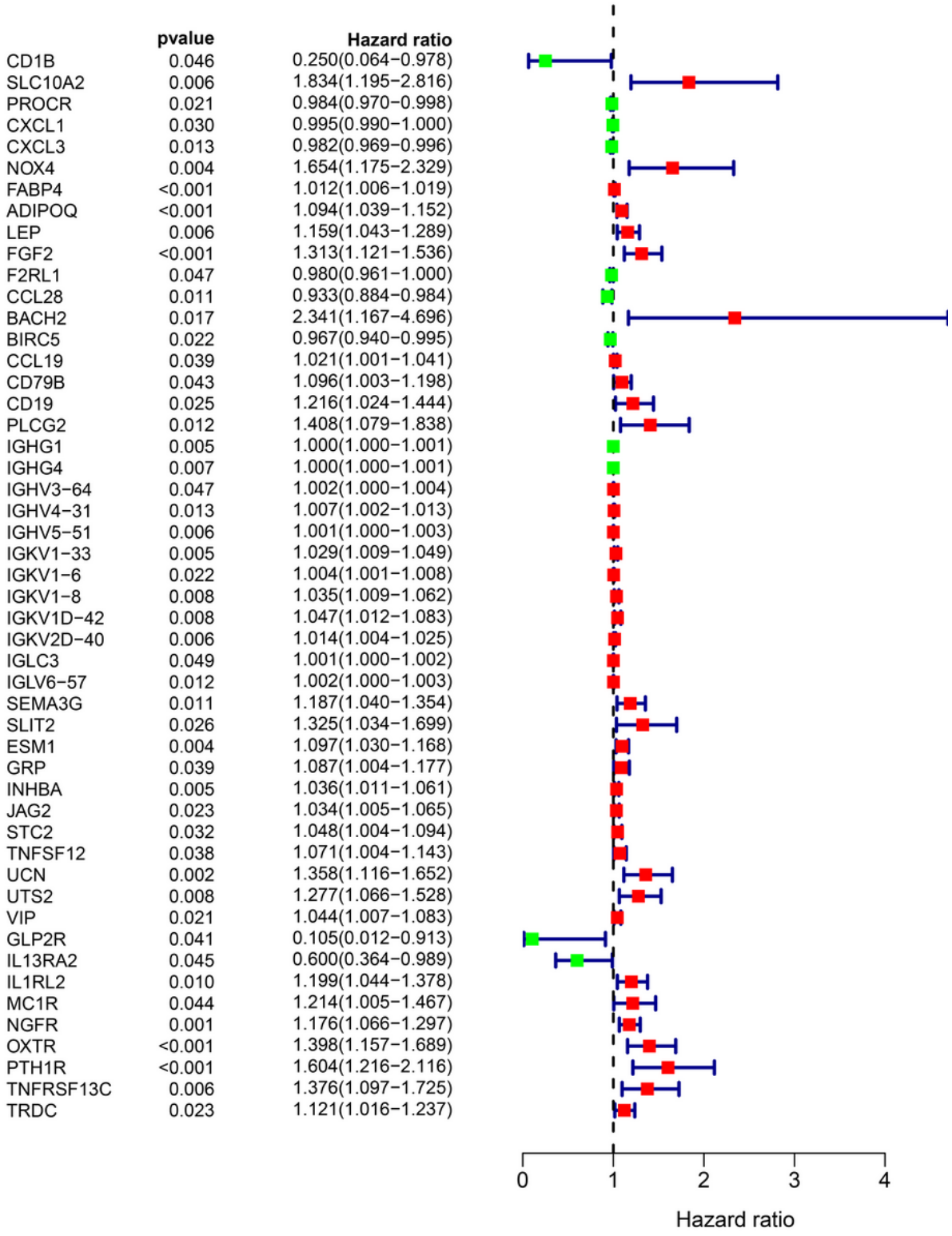



**Figure 2**

Heat map (A) and volcano map (B) showing differential expression of immune genes in colorectal cancer. Red: indicates upregulated immune gene, green: indicates immune genes with suppressed expression.




## Figure 3

Prognosis-related immune genes in colorectal cancer. Red: indicates high HR genes, green: indicates low HR genes.

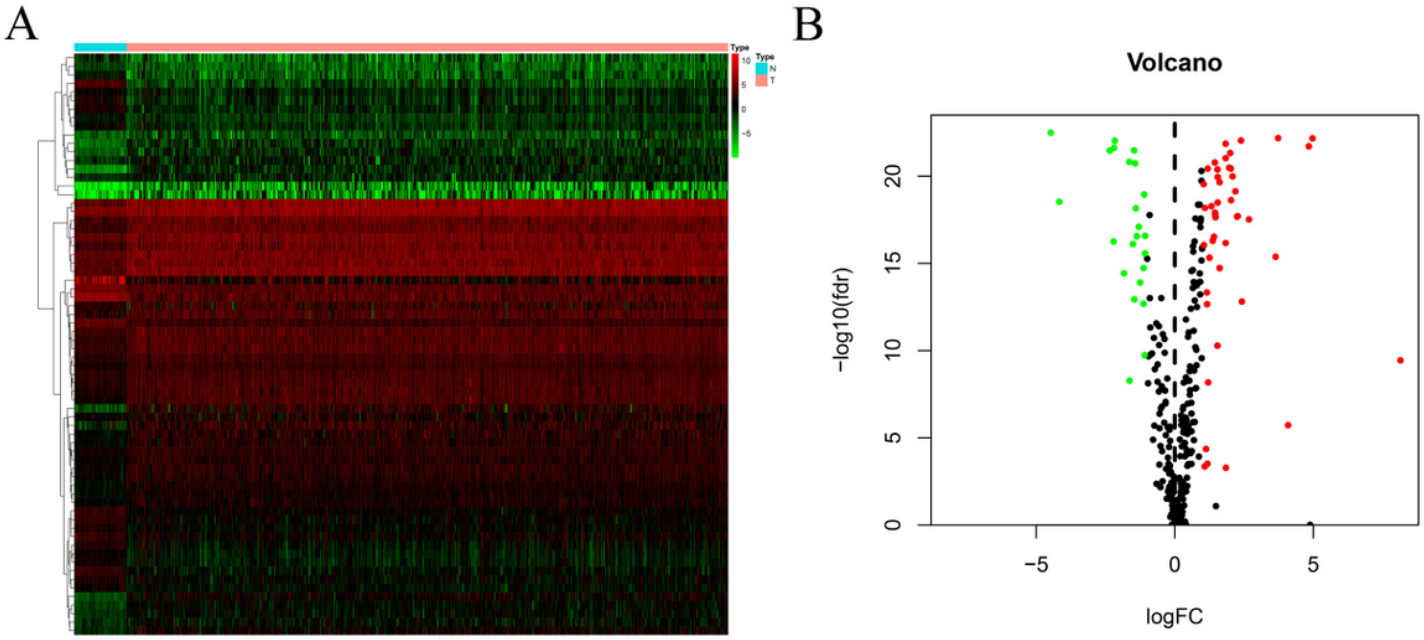
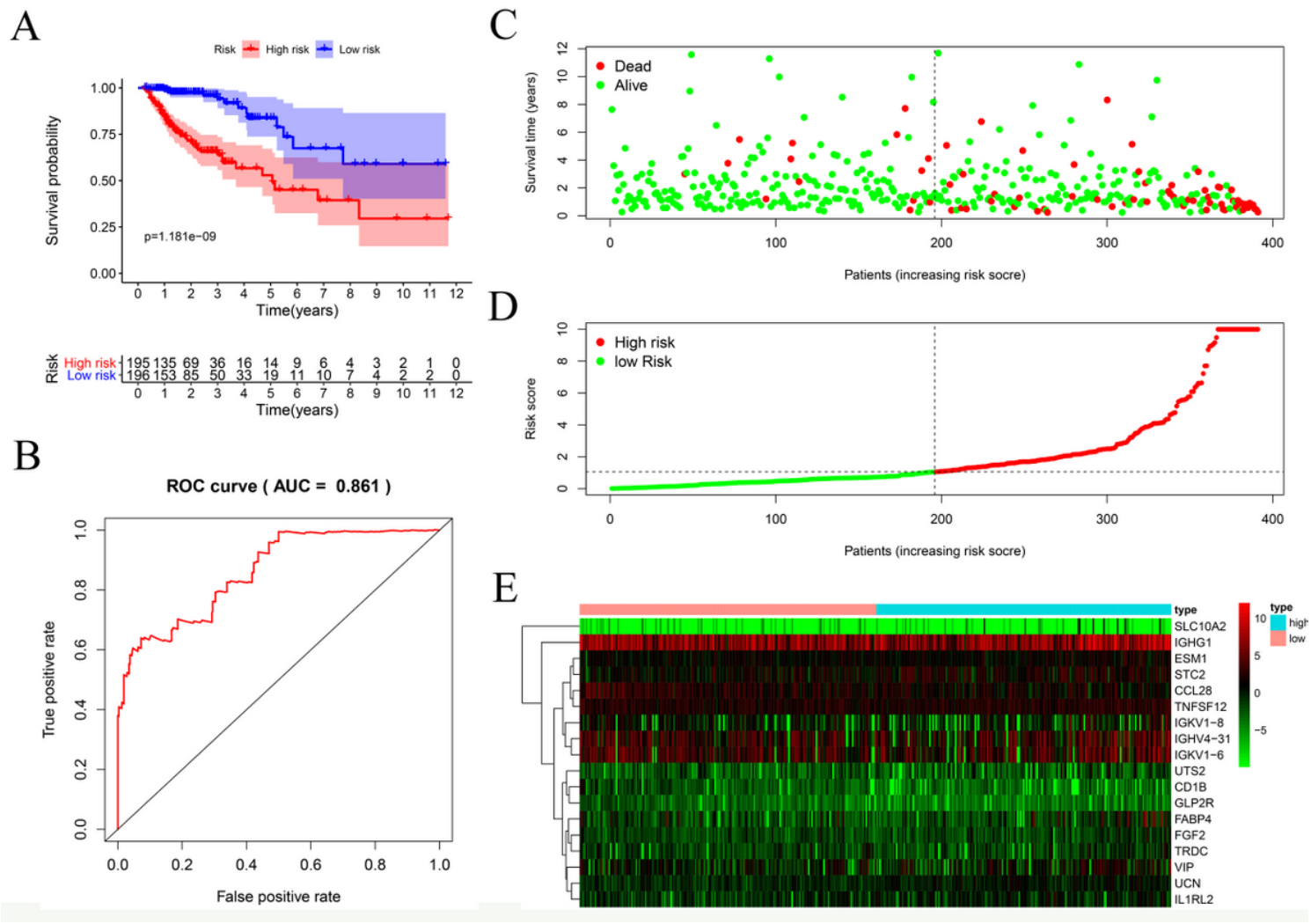


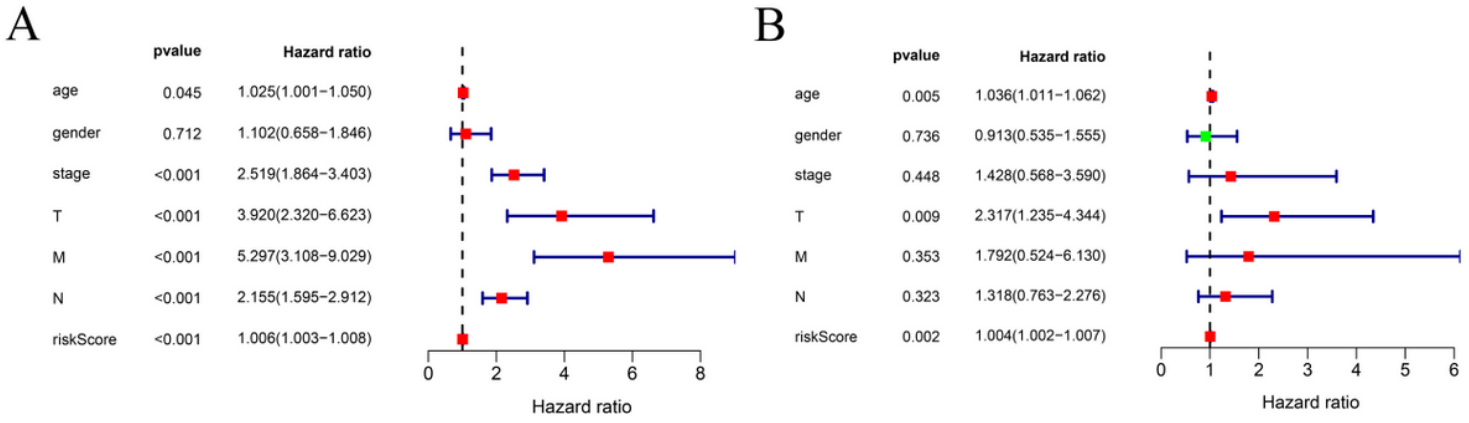

**Figure 4**

Heat map (A) and volcano map (B) showing differential expression of transcription factors in colorectal cancer. Red: shows up-regulated transcription factor, green: indicates down-regulated transcription factors.



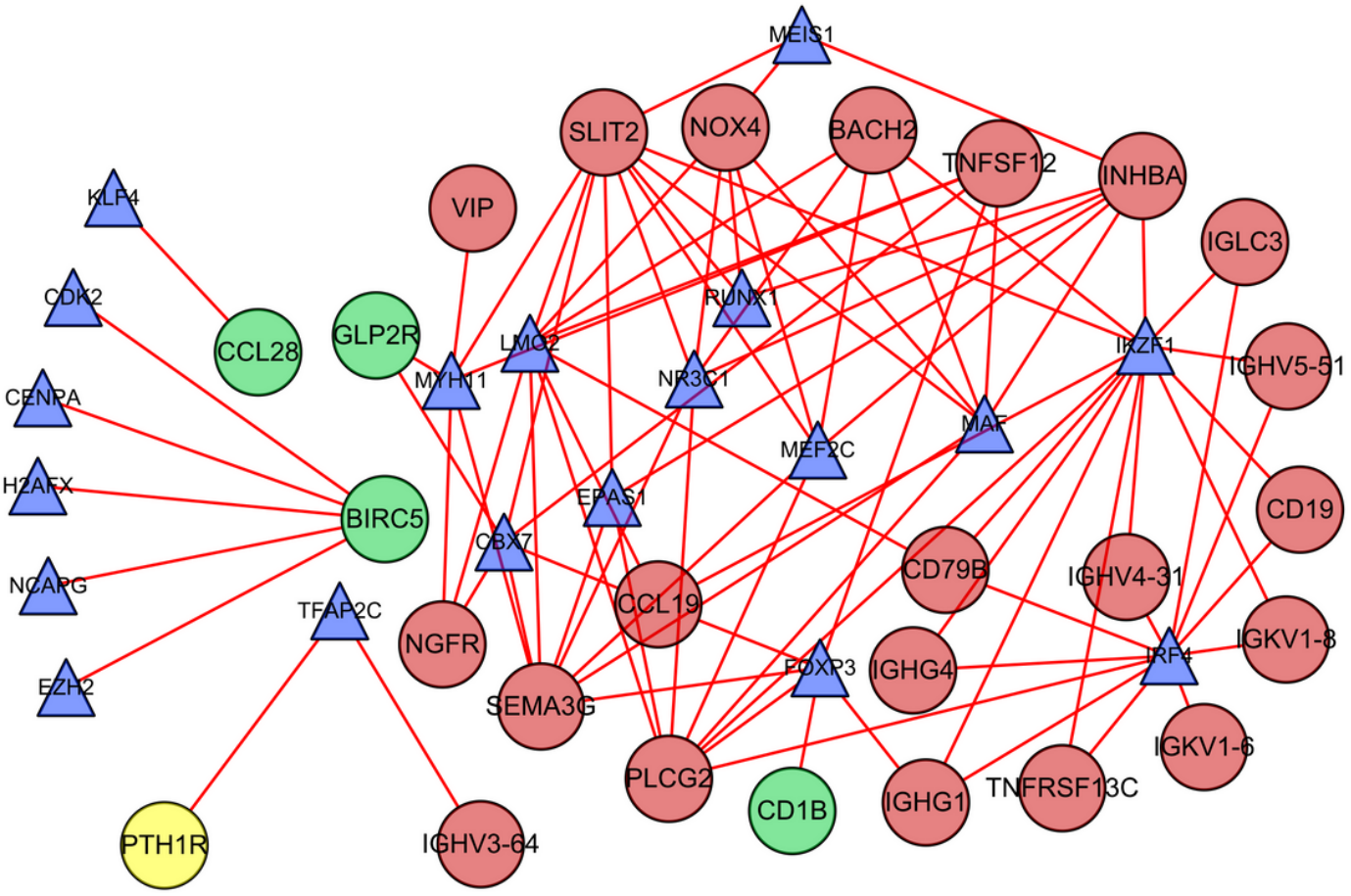

## Figure 5

Validation of the immune gene prognosis model for colorectal cancer. (A) Survival curve of prognostic model, red: shows high-risk group, blue: indicaties low-risk group, (B) ROC curve of the prognostic model, AUC indicates the area under the curve, (C) survival state graph of the prognostic model, (D) The risk curve of the prognostic model, (E) The risk heat map of the prognostic model.



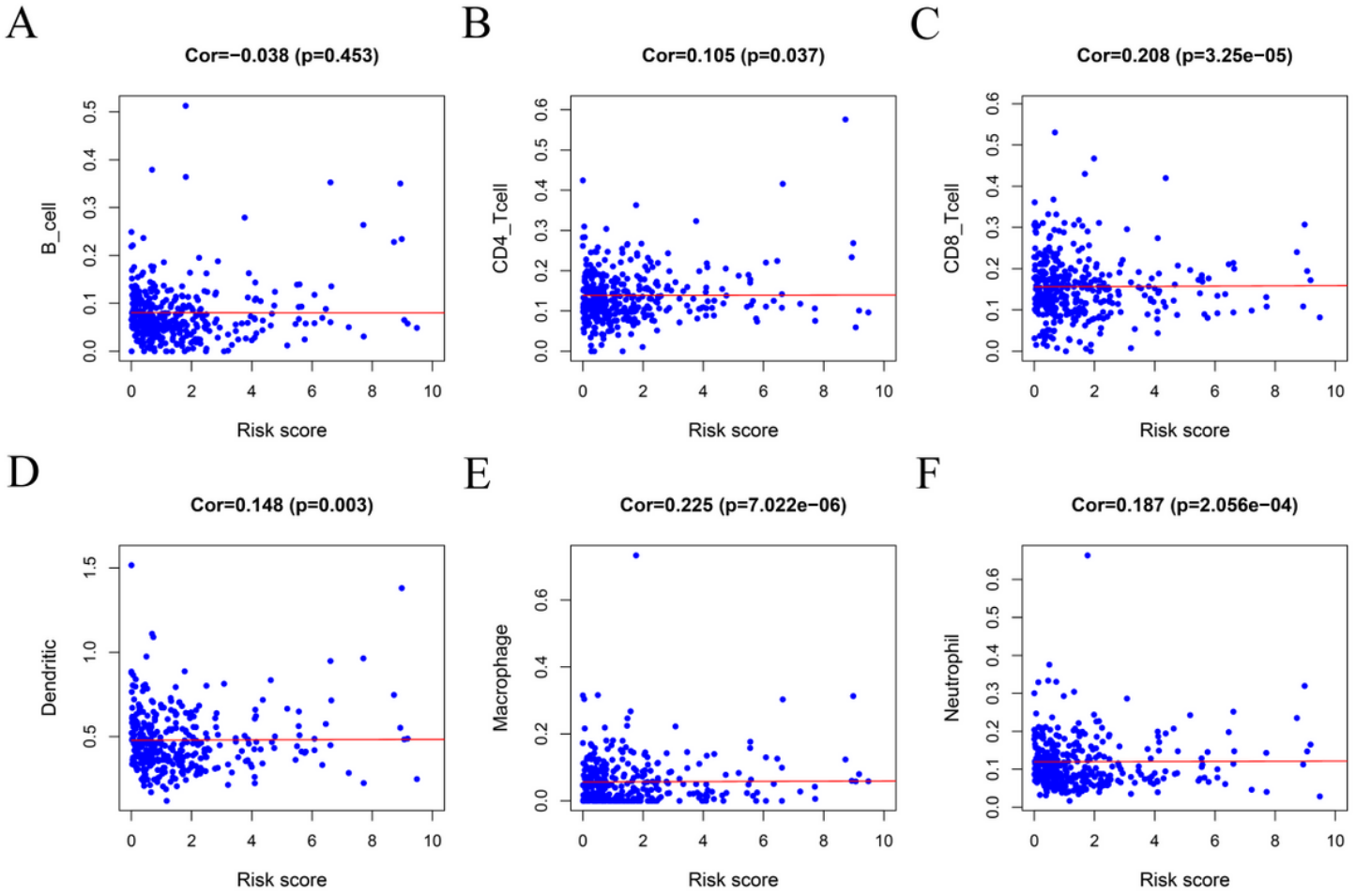

## Figure 6

Immune gene prognosis model and independent prognostic analysis of clinicopathological characteristics. (A) Single factor independent prognostic analysis, (B) Multivariate independent prognostic analysis. Red: indicates the hazard ratio (HR)>1, green: indicates the hazard ratio <1.




## Figure 7

The interaction network of transcription factors and immune genes. Circles: indicate immune genes, triangles: indicate transcription factors, red: indicate up-regulated genes, green: indicate down-regulated genes.




## Figure 8

Correlation analysis of the prognostic model risk score and colorectal cancer immune cells. (A) The correlation between risk score and B cells, (B) The correlation between risk score and CD4-T cells, (C) The correlation between risk score and CD8-T cells, (D) The correlation between risk score and dendritic cells, (E) The correlation between risk score and macrophages, (F) The correlation between risk score and neutrophils.